PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 0500	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IN2004/000432	International filing date *(day/month/year)* 30.12.2004	Priority date *(day/month/year)* 04.01.2004
International Patent Classification (IPC) or national classification and IPC INV. A61K39/12 C12N15/861 C07K14/18		
Applicant NATIONAL INSTITUTE OF IMMUNOLOGY		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.08.2005	24.04.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IN2004/000432

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-40	as originally filed

Claims, Numbers

1-20	received on 12.09.2005 with letter of 08.09.2005

Drawings, Sheets

1/5-5/5	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1, 4, 10
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10-20

because:

☒ the said international application, or the said claims Nos. 10-20 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-20
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

The present application relates to vaccines for Japanese Encephalitis virus (JEV), consisting of prM and secreted envelope (Es) protein delivered by a recombinant adenoviral vector.

Re Item I

Basis of the report

Claims 1, 4 and 10 have been amended to include a reference to accession number 04121701, which does not fulfil the requirements set out in Rule 13*bis*3(a) PCT. Amended claims 1, 4 and 10 are not allowable under Article 19 PCT, since the subject-matter of said claims may not be directly and unambiguously derived from the application as originally filed. Added subject-matter has not been taken into consideration.

The amendment of claims 4 and 10 by including a reference to 'JEV Es protein prepared by the method of claim 1' finds a basis in the examples of the present application and has thus found to be allowable.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 10-20 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: KAUR RUPINDERJEET ET AL: "Plasmid DNA immunization against Japanese encephalitis virus: immunogenicity of membrane-anchored and secretory envelope protein." THE JOURNAL OF INFECTIOUS DISEASES. 1 JAN 2002,

vol. 185, no. 1, 1 January 2002 (2002-01-01), pages 1-12

D2: US-A-5 494 671 (LAI ET AL) 27 February 1996 (1996-02-27)

D3: JAISWAL SMITA ET AL: "Replication-defective adenoviral vaccine vector for the induction of immune responses to dengue virus type 2." JOURNAL OF VIROLOGY, vol. 77, no. 23, December 2003 (2003-12), pages 12907-12913

NOVELTY (Article 33(2) PCT)

The prior art discloses vaccines for inducing an immune response directed against secreted JEV envelope protein: **D1** discloses a DNA vaccine protecting mice from lethal challenge with JEV, encoding JEV prM and either membrane anchored E protein (Ea) or Es protein (D1, table 2); **D2** shows that vaccinia virus comprising C-terminally truncated JEV E protein without prM induces a protective immune response in outbred mice (D2, table III), as well as neutralising antibodies in a number of outbred and inbred mouse strains (D2, table IV)

D3 discloses a recombinant adenovirus comprising a gene encoding Dengue 2 virus Es protein and demonstrates the induction of an immune response by said adenovirus (D3, fig. 1, 4, 5).

The prior art does not disclose an recombinant adenovirus comprising JEV Es and prM protein and its use as a vaccine, therefore the subject-matter of claims 1-20 appears novel.

INVENTIVE STEP (Article 33(3) PCT)

D1 is considered to represent the closest prior art for the vaccine of the present application, and said document disclose DNA vaccine compositions encoding JEV Ea or Es protein and prM (D1, p. 2, right-hand column), capable of inducing a protective immune response against challenge with wild-type JEV (D1, table 2).

From this the vaccine of the present application differs in that JEV Es and prM are delivered by an adenoviral vector.

The problem to be solved by the present invention may therefore be regarded as the provision of a further vaccine for JEV for inducing a protective immune response.

The solution is the provision of the adenovirus comprising JEV Es and prM.

The use of adenoviral vectors to develop vaccines for a flaviviral Es protein is known from

D3 and the skilled person would contemplate using adenoviral vectors to deliver Es from other flaviviruses, such as JEV. Furthermore, it has been demonstrated that JEV Es induces a stronger immune response than JEV Ea. However, in D2 as in other documents disclosing improved immunogenicity of the Es protein over Ea protein, said E proteins have been used in absence of prM.

D1 indicates that prM is necessary for correct processing and folding of the E protein, and teaches the skilled person that, contrary to D2, JEV Ea and Es protein linked to prM administered as a DNA vaccine have similar capacity of inducing an immune response in mice, both providing significant protection against challenge with wild type JEV (D1, table 2).

The skilled person would thus have concluded that there is no intrinsic lack of immunogenicity of Ea, and would have assumed that adenoviral vectors comprising JEV Ea or Es protein linked to prM would be equally capable of inducing a protective immune response.

Only after testing both JEV Ea and Es proteins in combination with prM in adenoviral vector, the advantages of the JEV Es encoding constructs became apparent: (i) the construct comprising JEV Ea protein resulted in lower virus titers produced, and (ii) despite similar overall levels of antibody and cytotoxic T cell response induced by the JEV Ea-prM and Es-prM adenovirus vaccines, the latter was superior in inducing neutralizing antibodies and protection against challenge with wild-type JEV.
In view of D1, the skilled person would not have anticipated this effect, hence an inventive step may be acknowledged for the subject-matter of the present set of claims.

INDUSTRIAL APPLICABILITY (Article 33(4) PCT)
For the assessment of the present claims 10-20 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the

manufacture of a medicament for a new medical treatment.

CLARITY (Article 6 PCT)

Claim 1 relates to a plasmid 'pMEs' defined by an arbitrary chosen name, which is meaningless to third parties and does not clearly define the product. pMEs does not seem to relate to **every** plasmid comprising Es and prM, since it is indicated that a specific combination of restriction enzymes is required to obtain the Es and prM cDNA for subsequent cloning in the right orientation.

A recombinant vector may be characterised by (1) its DNA sequence, (2) a combination of parameters and properties, (3) a deposit of a micro-organism in which the vector is present or (4) the composition of its sub-parts, provided said sub-parts have a clear meaning to the person skilled in the art.

Furthermore, the term RAdEs is ambiguous, since it seems to be the abbreviation of adenovirus and secreted envelope protein, whereas the invention seems to be restricted to an adenoviral vector comprising **JEV prM and Es** encoding nucleic acid sequences.

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Claims:

1. A method of preparing a recombinant adenovirus (RAdEs) vaccine - The Accession Number 04121701 - to protect against Japanese encephalitis virus (JEV) infection, wherein the said vaccine produces secretory envelop protein (Es) of JEV, said method comprising steps of:
   a. digesting plasmid pMEs with restriction enzymes *Kpn* I and *Bam* HI to obtain cDNA encoding JEV proteins prM and Es,
   b. ligating the cDNA to adenovirus shuttle plasmid pShuttle digested with restriction enzymes *Kpn* I and *Hind* III at the *Kpn* I end,
   c. filling nucleotides at the free *Bam* HI and *Hind* III ends with T4 DNA polymerase to create blunt ends,
   d. ligating the blunt ends together to yield shuttle plasmid pSEs with JEV cDNA encoding the proteins prM and Es,
   e. digesting the shuttle plasmid pSEs with restriction enzymes I-*Ceu* I and PI-*Sce* I to obtain expression cassette containing the JEV cDNA together with the CMV promoter/enhancer and BGH polyadenylation signal,
   f. ligating the digested shuttle plasmid with I-*Ceu* I and PI-*Sce* I digested adenovirus plasmid pAdeno-X to generate plasmid pAdEs containing Es expression cassette,
   g. digesting the plasmid pAdEs with *Pac* I,
   h. transfecting the monolayers HEK 293 cells with digested plasmid pAdEs for about one week, and
   i. obtaining the recombinant virus RAdEs vaccine. The Accession Number 04121701.
2. A method as claimed in claim 1, wherein the transfection is at about 37^0C temperature.
3. A method as claimed in claim 1, wherein the JEV proteins are under the control of human CMV IE promoter/enhancer.

AMENDED CLAIMS [ARTICLE 19]

4. A recombinant adenovirus (RAdEs) vaccine comprising JEV Es protein prepared by method of claim 1 having Accession No. 04121701, optionally along with pharmaceutically acceptable additives.
5. A vaccine as claimed in claim 4, wherein the vaccine produces secretory envelope protein of JEV.
6. A vaccine as claimed in claim 4, wherein the vaccine protects against Japanese encephalitis virus (JEV) infection.
7. A vaccine as claimed in claim 4, wherein the vaccine is effective by intra-muscular route of administration.
8. A vaccine as claimed in claim 4, wherein the additives are selected from a group comprising alum, gelatin and thiomersal.
9. A plasmid pAdEs of SEQ ID No. 1.
10. Use of a pharmaceutically effective amount of recombinant virus RAdEs vaccine comprising JEV Es protein prepared by method of claim 1 having Accession No. 04121701, optionally along with additive(s) to the subject in need thereof for Japanese encephalitis virus (JEV) infection.
11. Use as claimed in claim 10, wherein the method shows 100% efficacy.
12. Use as claimed in claim 10, wherein the method helps protect subject against encephalitis.
13. Use as claimed in claim 10, wherein the subject is animal.
14. Use as claimed in claim 10, wherein the subject is a human being.
15. Use as claimed in claim 10, wherein the immunization activates both humoral and cell-mediated immune response.
16. Use as claimed in claim 10, wherein the humoral response to the vaccine comprises IgG1 type of antibody.
17. Use as claimed in claim 10, wherein the method leads to high amount of IFN-gamma secretion.
18. Use as claimed in claim 10, wherein immunization leads to moderate levels of IL-5 synthesis.
19. Use as claimed in claim 10, wherein increased amount of RAdEs leads to higher immune response.

AMENDED CLAIMS [ARTICLE 19]

20. Use as claimed in claim 10, wherein the method is more effective than the commercially available vaccines.